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Antimicrobial resistance of *Escherichia coli* found in intestinal tract of *Oreochromis niloticus*

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Abstract

Antimicrobial resistance (AMR) is an emerging public health problem worldwide. Agricultural practices have been suggested as one of the main contributors to the emergence and transmission of AMR worldwide. Aquatic animals like fish have an important role to play in the emergence and transmission of AMR strains. *Escherichia coli* is commensal in gastrointestinal tracts of many animals including fish, and is an indicator organism and opportunistic pathogen. It has been listed as one of the important bacteria involved in the emergence and spread of antimicrobial resistance factors. The objective of this study was to investigate the AMR patterns of *E. coli* isolated from the intestinal tract of fish within the Lake Victoria basin. A total of 60 *Oreochromis niloticus* fish were sampled from cages, wild and earthen ponds. Distal gastrointestinal tract was recovered, crushed and streaked on violet red bile agar, incubated at 37 ^{O}C for 24 hours. The isolates confirmed biochemically as *E. coli* were screened for antibiotic susceptibility against 10 commonly used antibiotics using disc diffusion method. Eighty three percent of the sampled fish were positive for *E. coli*. Generally, isolates were susceptible to ciprofloxacin, gentamicin, cephalexin, ceftriaxone and nitrofurantoin (100%); tetracycline, sulfamethoxazole-trimethoprim (81.8%) and nalidixic acid (72.7%). However, most of them were resistant to ampicillin and erythromycin (72.7%). The results show that fish are reservoirs of *E. coli,* which is resistant to some antibiotics. The factors responsible for the observed resistance patterns need to be followed up with more research. Good aquaculture practices, surveillance and monitoring of resistant bacteria will help in understanding the problem of AMR.

Key words: Antibiotics, antimicrobial resistance, *Escherichia coli*, fish

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Introduction

Antimicrobial resistance (AMR), an emerging public health problem worldwide is more pronounced in resource constrained countries (Blomberg, 2008; Okeke *et al.,* 2005, 2007). The global health security agenda has listed AMR as number one target in a bid to improve health and wellbeing. *Escherichia coli*, a rod shaped Gram negative facultative anaerobe is among the priority organisms that should be targeted for antimicrobial resistance. *Escherichia coli* occurs as normal flora in the gut of many animals (including humans), and has been associated with diseases in humans and other animals, displaying its potential for transmission of resistance factors across species. It is used as an indicator organism for both food and environmental fecal contamination. Agricultural practices have been suggested to play an important role in the emergence and spread of AMR owing to the fact that antimicrobial agents are used as growth promoters and for prophylaxis in livestock production (Krumperman, 1983; Kemper, 2008).

Currently, there is a campaign to increase aquaculture production in countries like Uganda; hence, the need for more intensive production systems that may involve prophylactic application of antibiotics. The use of antimicrobial agents in aquaculture for prophylaxis and growth promotion has been documented (Durborow and Francis-Floyd, 1996; Musefiu and Olasunkanmi, 2015; Serrano, 2005; Tiamiyu *et al*., 2015), but there is no record regarding the use of antimicrobial agents in aquaculture systems in Uganda. The unanswered question remains the situation of antimicrobial use in aquaculture and its impact on the emergence, maintenance and transmission of AMR. Water bodies serve as meeting points for humans, terrestrial and aquatic animals, and could play an important role in the emergence, maintenance and spread of AMR (Biyela *et al.*, 2004; Martinez, 2009; Wellington *et al*., 2013; Marti *et al*., 2014). Fish can give a good representation of microbial interactions in water bodies. Nile tilapia (*O. niloticus*) is the most cultured and widely consumed fish species in Uganda, and its economic importance cannot be underestimated. That notwithstanding, tilapia has *E. coli* in the GIT that could contribute to the emergence, maintenance and spread of antimicrobial resistance. The objective of this study was to isolate *E. coli* from gastrointestinal tracts of *O. niloticus* and test AMR against the most commonly used antimicrobial agents.

Materials and methods

Sixty *O. niloticus* were collected between November 2015 and March 2016 from three groups of environments based on husbandry practices from the Lake Victoria basin in Uganda. These environments included intensive cage culture on Lake Victoria, wild fish from the same lake and from earthen ponds in the districts of Mukono, Mpigi, Wakiso and Buikwe in central Uganda. Fish were transported in source water to the Central Diagnostic Laboratory, College of Veterinary Medicine Animal Resources and Biosecurity (COVAB), Makerere University in Uganda.

In the laboratory, fish were humanely put to rest and dissected to remove the hind gut which was aseptically crushed and the contents used as inoculum on violet red bile agar (VRBA). The set up was incubated at 37 °C for 24 hours. The resulting colonies were inoculated on fresh

VRBA and subjected to temperature incubation at 44 °C for 24 hours. Isolates were confirmed as *E. coli* when found Gram negative short rods, positive for methyl red and indole tests; and negative for Voges-Proskauer, oxidase and citrate tests.

Antimicrobial susceptibility testing was performed using the disc diffusion (Kirby-Bauer) test (Bauer *et al.*, 1966). Fresh colonies on VRBA were picked using a sterile wire loop, suspended in normal saline and turbidity adjusted to 0.5 McFarland standard (CLSI, 2014). The adjusted bacteria suspension was then surface spread on Mueller Hinton agar (Difco) and antibiotic impregnated discs applied. The antibiotics that were used are Ampicillin-10µg (AMP), Erythromycin- 15 µg (ERY) Tetracycline -300 µg (TET), Gentamicin - 5 µg (Jorgensen and Turnidge, 2015), Cephalexin-30 µg (CLSI, 2014), Ciprofloxacin-5µg (CIP), Ceftriaxone – 30 µg, Nitrofurantoin-30 µg (F), Sulphurmethaxazole-trimethoprim (SXT), 1.25 µg 23.5 µg Nalidixic acid – 30 µg (NA). These were selected based on the Uganda Ministry of Health clinical guidelines, CLSI and the list of essential medicines in veterinary practice. Five different antibiotic discs were applied on each plate and incubated at 37 °C for 24 hours. The inhibition zone was measured and scored as susceptible, intermediate or resistant following CLSI guidelines.

Results and discussion

A total of 50 *E. coli* were isolated from fish in the selected environments, i.e. eight from wild fish from Lake Victoria, 11 from caged fish (on Lake Victoria) and 31 from earthen ponds. Twenty two of these isolates were then selected for antibiogram, giving results in Tables 1 and

Table 1. General antibiogram for all the isolates and all antimicrobial agents used

	Antibiotic agents (N= 22)									
	AMP	ERY	TET	GEN	CL	CIP	CRO	F	SXT	NA
S, n (%)	6 (27.3)	6 (27.3)	18 (81.8)	22 (100.0)	22 (100.0)	22 (100.0)	22 (100.0)	22 (100.0)	18 (81.8)	16 (72.7)
R, n (%)	16 (72.7)	16 (72.7)	4 (18.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (18.2)	6 (27.3)

S = Susceptible, R = Resistant

2. The highest resistance was observed for AMP, ERY, NA, TET and SXT, in descending order, respectively. Complete susceptibility was observed for GEN, CL, CIP, CRO and F. Although the sample size was small, this study presents the first report on antimicrobial susceptibility of *E. coli* from fish and its public health implications within the Lake Victoria basin. Differences were observed relating source of the sampled Nile tilapia and antimicrobial resistance profiles (Table 2). Generally, fish from wild capture in Lake Victoria had bacteria that were 100% resistant to AMP, ERY, TET and SXT, higher than that reported in tilapia (Rocha *et al.*, 2014) and other fish species elsewhere (Kumaran *et al*, 2010; Bolarinwa *et al*, 2011; Ryu *et al.*, 2012; Musefiu and Olasunkanmi, 2015). The observed AMR levels elsewhere could be explained by the fact that different levels of application of antibiotics in aquaculture, which is not documented in Uganda. The observation in this study could suggest a high level of aquatic ecosystems contamination by human activities. Two theories have been advanced to explain the emergence of resistance in wild waters, i.e. resistant bacteria contaminating water from human or livestock sources; or antibiotic residues escaping into the water exposing the resident bacteria to low levels of antimicrobial agents overtime (Costanzo *et al.*, 2005). Transfer of antimicrobial resistant bacteria to wild water due to sewage contamination has been reported in Australia (Costanzo *et al.*, 2005). Bearing in mind the low toilet coverage in most of the settings along the lake shores of Lake Victoria (Kabatereine *et al.*, 2014; Loewenberg, 2014), contamination of the aquatic ecosystem by human activities is highly probable. The resisted

Table 2. Antibiogram based on source of the isolates

	AMP	ERY	TET	GEN	CL	CIP	CRO	F	SXT	NA
Lake (N=4)										
Resistant (%)	100	100	100	0	0	0	0	0	100	0
Susceptible (%)	0	0	0	100	100	100	100	100	0	100
Cage (N=4)										
Resistant (%)	0	0	0	0	0	0	0	0	0	100
Susceptible (%)	100	100	100	100	100	100	100	100	100	0
Pond (N=16)				0						
Resistant (%)	85.70%	85.70%	0	100	0	0	0	0	0	28.6
Susceptible (%)	**14.3**	**14.3**	100		100	100	100	100	100	71.4

Source: Antimicrobial agents

antibiotics are those commonly used for treatment of various ailments in humans, thus strongly supporting the theories of introduction into the aquatic ecosystems from human associated sources. Studies along the Nakivubo channel in Uganda revealed presence of antibiotic residues in sediments, water and soil (Owino, 2014; Fuhrimann *et al*., 2015) with very low levels of gentamicin and ceftriaxolne which we also found were effective in this study.

Bacteria from cage fish on Lake Victoria were susceptible to all antibiotics, except NA (Table 2) This observation could be explained by the fact that the cage farms sampled were situated in areas with reduced human activity; while the wild Nile tilapia was captured at the shorelines with increased human activity, thus further underscoring the influence of human activities in the emergence and spread of antimicrobial resistant bacteria to Nile tilapia. Bacteria isolated from earthen domestic ponds were 85.7% resistant to AMP and ERY; and 28.6% resistant to NA (Table 2) but susceptible to other antibiotics used in the study.

The highest level of resistance observed against AMP and ERY has a direct public health implication, as these drugs are commonly prescribed for bacterial infections in health facilities (Mbonye *et al*., 2016). This implies that once such strains cross back into the general population, they will be difficult to treat. The risk of antibiotic use in aquaculture to human health has been reviewed (Sapkota *et al*., 2008). In addition, some of the isolated bacteria were resistant to more than one family of antibiotics, signaling the occurrence of multidrug resistance in Nile tilapia. Considering that Nile tilapia has both economic and nutritional values, and that handlers are not aware of the possibility of such bacteria, puts their health and that of the community at risk. *Escherichia coli* has been reported to develop both genome and plasmid mediated resistance that can be shared with compatible bacteria within the aquatic environments through conjugational or transformational events (Witte, 2000; Alexander *et al*., 2010). This implies that presence of the antibiotic resistant bacteria in the aquatic ecosystems that are resistant to commonly used antimicrobial agents poses a risk to AMR emergency in both terrestrial and aquatic animals. Studies elsewhere have revealed that *E. coli* strains in aquatic ecosystems constantly contaminated with antibiotics are not only resistant but greatly pathogenic (Hamelin *et al*., 2007).

Application of animal excreta to fertilize earthen ponds by small holder framers has been reported as the main source of bacterial contamination into the ponds (Dang and Dalsgaard, 2012). However, the resistance pattern observed in pond isolates in this study was not supportive as the ponds from where the samples were collected were not using this technology. In addition, the resistance observed in ponds was against antibiotics not commonly used in veterinary practice in Uganda further pointing to human sources. This study, therefore, confirms the occurrence of antibiotic resistant *E. coli* in Nile tilapia in Uganda, suggesting the need to investigate further both water and other aquatic animals in order to understand the emergence and transmission patterns of AMR. Such broad based findings will help guide policy involving regulation of antimicrobial resistance in aquatic ecosystems that pose a great risk to humans and livestock.

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